REMARKS

Claims 20 to 39 were pending in this application prior to entry of the above amendments. Claims 20, 22, and 23 have been amended, and claims 31 to 39 directed to a nonelected invention and claim 25 have been cancelled and replaced by claims 40 to 50. Because the amended claim set comprises fewer than 20 claims, 2 of which are independent, no new claim fees are due or submitted herewith. However, applicants are two months late in replying to the above-referenced outstanding office action, so an Extension of Time Request and fee are due and presented with documents accompanying this amendment.

This invention relates to agents such as EtxB which are identified and employed to treat allergic and/or hypersensitivity conditions. These remarks address issues raised by the Examiner in the order presented in the Office Action and are cross-referenced to the numbered items in the Action.

2/3. Applicants have inserted a related application paragraph and an abstract to the specification as requested by the Examiner. The abstract is identical to the one that appeared on the cover sheet of the PCT application.

4. Applicants have changed the claim 23 spelling of "signalling" as requested by the Examiner.

6-9. Claims 20 to 30 were rejected under 35 U.S.C. § 112 as being indefinite in the recitation of "mutants or derivatives thereof" and "modulates" in claim 20. The claim has been amended in response to this rejection. Amended claim 20 refers to mutants and derivatives of CtxB, Etx and EtxB which display the parametric changes as indicated, functionally limiting the claim. Claim 25 was, therefore, cancelled as amended claim 20 incorporates its limitation. Support for the added parameters may be found in

the specification on page 33, line 9 to page 34, line 15. Applicants submit that claim 22 is not limited to ANY GM-1 binding agent but only to those derivatives of Ctx, Etx, or EtxB which display the particular parameter changes as set out in claim 20. New claims 40 to 42 particularly point out embodiments using EtxB derivatives or mutants having the functional properties set out in the dependent and parent claims, and the use of Ctx, Etx, or Etx B themselves. New claims 41 and 42 are especially allowable over the rejection, as they particularly point out known molecules.

Claim 20 and subsequent dependent claims have been amended to that the term "modulate" has been retained in part (b) of claim 20, but more specific language has been used in part (b) to define the parametric changes in IgE levels and associated Th2 cytokine levels such as IL-4 levels. Applicants submit that the term "ganglioside associated activity" has been defined on page 15, lines 7-9. There is also an internal definition of this term in amended claim 20 because the term has now been further defined with respect to specific parameter changes.

As indicated above, the term "mutants and derivatives thereof" have also been defined with respect to these parametric changes. In view of the repercussive effect of claim 22 on claim 20 and claim 21, the term "agent" includes agents that bind to GM-1 and agents that fail to bind to GM-1. These agents are now defined functionally in claim 20 with respect to their specific effects on IgE antibody levels and Th2 cytokine levels (such as IL-4) levels. Any agent that does not demonstrate this effect does not fall within the scope of the term "agent". Accordingly, as discussed below, a mutant of EtxB, such as G33D, which does not demonstrate these effect, will not fall within the scope of the claim. Applicants submit that it would be routine for the skilled person to identify the agents which fall within the scope of claim 20 simply by determining the effect of the agent on IgE levels and associated Th2 cytokine levels. Applicants believe that a limitation of claim 20 to GM-1 binding agents would be both unnecessary and unduly limiting.

10-12. Claims 20 to 30 were rejected under 35 U.S.C. § 103(a) as being obvious over *Nashar, et al., P.N.A.S. USA 93*: 226-230, 1996; Yamamoto, *et al., J. Exp. Med. 185*: 1203-1210, 1997; and Kim, *et al., J. Immunology 160*: 1198-1203, 1998. The rejection is respectfully traversed as Applicants believe the rejection is based on a hindsight reading of the references with the knowledge of Applicants' dicslosure.

It is well known that the administration of EtxB and other homologues can modulate the immune response away from the production of Th1 cytokines such as IFN-γ and interleukin 2 (IL-2) and towards the secretion of Th2 cytokines such as IL-4, IL-10 and IL-13 (see Nashar, *et al.*, and other papers by the same investigators in *P.N.A.S. USA 93*: 223-226, 1996; *Int. Immunol. 8*: 731-736. 1996; and *Immunol. 91*: 572-578, 1997, attached hereto). IFN-γ is the classical Th1 cytokine while IL-4 is the classical Th2 cytokine. This "immune deviation" is also the basis of the disclosure in WO 97/02045 and has been shown to be effective in the treatment of autoimmune diseases.

The experimental results in WO 97/02045 would suggest that GM1 binding agents, such as EtxB, would not find use in the treatment of allergic conditions and/or hypersensitivity conditions since such conditions involve IgE, the production of which is generally accepted to be promoted by IL-4 (see for example, pages 22.2 -22.4 of "Immunology" 4th Ed (Roitt, Brostoff and Male, eds. 1996, a copy of which is enclosed). Thus, the teachings in WO 97/02045 would suggest that the administration of EtxB and other homologues would upregulate the production of IL-4 from Th2 helper cells which would then promote the production of IgE. Since IgE is known to act as a mediator in an allergic response, it would be counterintuitive to use an agent such as EtxB or its homologues in the prevention and/or treatment of an allergic response or to search for agents like EtxB or its homologues useful in the treatment of allergic responses. Thus, there was a clear technical prejudice in the art before the priority date of the present invention against using an agent such as EtxB to prevent and/or treat an allergic and/or hypersensitivity condition.

Applicants submit herewith further data to support the invention as set out in the amended claims. This data is set out in Annex I.[1] Importantly, this data demonstrates that, contrary to the generally accepted teachings (as set out in publications such as Yamamoto, *et al.*), the EtxB subunit does not promote IgE production. Significantly and surprisingly, Applicants have demonstrated for the first time that the EtxB subunit actually suppress the Th2 response as indicated by its effect on the suppression of IgE production.

It is clear from the teachings in the present application that a mucosa binding agent coupled to an allergen has been mentioned in relation to the treatment of allergy (see WO 95/01301 cited on page 7, lines 19-28). It is also clear from the teachings in the present application that other researchers such as Tamura, *et al.* (*Vaccine 15*: 225-229, 1997) have taken directly the protocol of WO 95/10301 and tested its efficacy in preventing allergy in a murine model of Type I allergy. They reported a significant lowering of IgE levels which are a strong predictor of efficacy but they cite data, following administration of EtxB coupled to ovalbumin, which shows that EtxB was NOT effective once IgE levels are established (i.e. EtxB coupled to ovalbumin was not effective in treating allergy). Thus, Tamura, *et al.*, point away from the present invention which shows that EtxB is an effective treatment in an ovalbumin (OVA) asthma model by suppressing the production of IgE antibodies even when it is not conjugated to an antigen. Furthermore, whilst Tamura et al teach that EtxB-OVA conjugates can prevent allergy, there is no disclosure of suggestion in Tamura et al that EtxB can work in the absence of a conjugated antigen.

In response to the specific objections raised by the Examiner, the disclosure in each document will be now be considered in turn.

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[1] The undersigned hereby declares that the statements presented in the Annex are Applicants' data transmitted via their British patent attorney, Dr. Charles Harding of D. Young, and Co., and are believed to be true; further that these statements are made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Nashar, *et al.* (1996) describes an assay method for measuring levels of various cellular cytokines and antibodies associated with an immune response. The disclosure in Nashar, *et al.*, relates to a comparison between EtxB and a mutant of EtxB (G33D) which does not bind to the GM-1 receptor. Nashar, *et al.*, teaches that:

> EtxB in comparison with EtxB (G33D) caused an increase in the proportion of B cells, many of which were activated (CD25+); the complete depletion of CD8+ T cells; an increase in activation of CD4+ T cells; and an increase in interleukin 2 (IL-2) and an increase in interferon gamma (IFN-γ; see abstract on page 226).

Although Nashar, *et al.*, teach that IFN-γ and IL-2 can be detected in the supernatants from cultures of EtxB and EtxB (G33D) with lymphocyte populations, no IL-4, IL-5 and IL-10 could be detected in either culture. Moreover, the teachings in Nashar, *et al.*, are confined to EtxB subunit and a mutant of the EtxB subunit. In fact, Nashar, *et al.*, suggest that commercial preparations of Ctx and CtxB or purified CtxB are strongly inhibitory of lymphocyte proliferation (see page 229, right column). Thus, there are conflicting teachings in Nashar, *et al.*, in relation to the effects of Ctx and Etx on lymphocyte proliferation.

It is also clear that Nashar, *et al.*, do not disclose or suggest that:
(i) either Ctx, CtxB, Etx or EtxB might play a role in an allergic response;
(ii) the specific changes/modulating in cytokine/antibody levels which occur in an allergic response; and/or
(iii) how an assay method might be developed to identify and agent which could induce such specific changes.

Yamamoto, *et al.* (1997) confirm the generally accepted wisdom in the art that agents like Ctx can induce increases in total and specific antigen specific IgE antibodies (see page 1206, col 1 and Table 2), and these increases are associated with IL-4 production (see page 1206, col 2 and Figure 3 and commentary on page 1207, col 1). Yamamoto, *et al.*, does not disclose or suggest that agents such as CtxB, Etx or EtxB could be used in the treatment of allergy. Indeed, the results in Yamamoto, *et al.*, point away

from the possible usefullness of agents such as Ctx and mutants thereof in the treatment of allergy because the results in Table 2 indicate that agents such as Ctx and mutants thereof actually promote the production of IgE antibodies which are known to be the cause of allergy.

In addition, even though the studies in Yamamoto et al mostly relate to the Ctx holotoxin, the para bridging pages 1207 and 1209 of this publication states that:

> Although CT and LT are both potent adjuvants, the molecules differ in terms of the nature of the CD4+ Th subsets induced and the profile, isotype and subclass of Abs induced. For example, CT induces adjuvanticity by promoting Ag-specific CD4+ Th2 type responses associated with high levels of IL-4 and IL-5 production with the provision of help for IgG1 subclass, IgE and s-IgA responses whereas LT promotes Th1- and Th2- type responses with high levels of IFN-g and IL-5 production and subsequent IgG1, IgG2a, IgG2b subclass and S-IgA Ab responses....

Thus, the teachings in Yamamoto et al (1997) confirms the generally accepted wisdom that:

(i) the adjuvant properties of Etx and Ctx has been examined; and

(ii) that Ctx triggers a Th2 dominated response to added antigens whereas Etx (LT) gives a more balanced response involving stimulation of Th1 and Th2 responses.

It is clear that the teachings in Yamamoto, *et al.*, are silent about the possible usefulness of agents such as CtxB, Etx, EtxB and/or mutants or derivatives thereof in the prevention and/or the treatment of allergic/hypersensitivity conditions and thus of the possible usefullness of setting up an assay method to identify agents such as these. By way of example, although Yamamoto, *et al.*, teaches that Ctx and mutants of Ctx actually promote the production of IgE level, the teachings in Yamamoto, *et al.*, are silent about the possible effects of the CtxB subunit both in the presence and/or absence of added and/or conjugated antigen.

In addition, the teachings in Yamamoto, *et al.*, in relation to Etx (also known as LT) do not disclose or suggest that subunits of the Etx family could be potential candidates for suppressing antigen specific IgE production. More importantly, Yamamoto et al do not suggest that the EtxB subunit would be less potent in inducing a Th2 response or that the EtxB subunit might actually suppress the induction of a Th2 response by suppressing IgE production.

It is also clear from the teachings in Yamamoto, *et al.*, and the findings of the present invention that the B subunits behave differently to the whole toxin or detoxified mutants as set out in Yamamoto, *et al.* In this respect, Applicants submit that a detoxified Ctx is not the same as a CtxB subunit because even though a detoxified Ctx will not have ADP-ribosylation properties, a detoxified Ctx subunit will have other properties besides ADP-ribosylation such as the presence of an ER localisation sequence which are not present in the CtxB subunit.

In view of the fact that:

(i) the conflicting teachings in Nashar, *et al.*, in relation to the effects of Ctx and Etx on lymphocyte proliferation; and

(ii) the teachings in Yamamoto, *et al.*, would discourage the skilled person from using Ctx or mutants thereof as possible agents in the treatment of allergy,

the skilled person would not have been motivated to combine the teachings of Nashar, *et al.*, and Yamamoto, *et al.*, as they would have received no inspiration from the citations to do so. Even if the skilled person were motivated to combine the teachings, the skilled person would not arrive at the claimed invention.

Kim, *et al.* (1998) relates to the mechanism by which CtxB promotes IgA mucosal immunity and oral tolerance through the upregulation of TGFb1 activity. Kim, *et al.*, do not disclose of suggest that agents such as CtxB, Etx or EtxB could be used in the treatment of allergy. Indeed, the Kim, *et al.*, paper only refers to the cholera toxin (Ctx) and the cholera toxin B subunit (CtxB). No mention is made of Etx or EtxB. In view of

the fact that Yamamoto, *et al.*, points away from the use of the cholera toxin (Ctx) in the treatment of allergy and hence points away from an assay method for identifying agents useful in the treatment of allergy, the skilled person would have no incentive to combine the teachings of Kim, *et al.*, with the teachings of Yamamoto, *et al.*

Applicants therefore believe that they have made a new and unobvious contribution to allergic and/or hypersensitivity therapies, and respectfully request allowance of the amended claims.

Respectfully submitted,

on 21 August 2001 by

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Marked Up Version of Amendments Required by 37 C.F.R. § 1.121

20 (Amended). An assay method for identifying an agent useful in the treatment of an allergic or hypersensitivity condition comprising:

(a) contacting a test agent with a ganglioside receptor, wherein the agent is not coupled to an antigen;

(b) determining whether the agent modulates a ganglioside associated activity by measuring a change in at least one parameter selected from the group consisting of: a [change] suppression in antigen specific IgE levels, a reduction in the production of Th2 associated cytokines; a change in antigen specific T-cell reactivity, a change in IgG levels, a change in IgA levels, and any combination thereof; and

(c) identifying the useful agent by observation of modulation of ganglioside associated activity.

22 (Amended). A method according to claim 21 wherein the agent is selected from the group consisting of [Ctx, Etx, CtxB, EtxB, and] mutants or derivatives thereof that bind to GM1.

23 (Amended). A method according to claim 20 wherein the agent has an effect on GM1 mediated intracellular [signalling] signaling events, but no GM1 binding activity.

ANNEX I

Sensitization

Mice were sensitized to ovalbumin (OVA) (10 µg/injection) adsorbed to 1.5 mg Al $(OH)_3$ by intra-peritoneal injections on day 1, 14 and 21. Control mice were immunized with PBS alone.

Challenge

Animals were challenged on days 27 and 28 by ultrasonic nebulization of 1% OVA (chicken OVA, grade V, Sigma Chemical Co., St. Louis, Mo.) diluted in sterile PBS. For this purpose, five mice were placed into a clear plastic box with a re- movable top (dimension 22 by 23 by 14 cm). The OVA solution was aerosolized into one end of the box with an ultrasonic nebulizer and a continuous pressure of 5psi. At the other end of the chamber were two small air holes to ensure a continuous cross-current of air flow.

Experimental groups (n=10/group)

Group 1: Sensitization negative control (PBS i.p.)
Group 2: Sensitization positive control (i.p. OVA; untreated)
Group 3: EtxB alone (i.p. OVA; i.n. EtxB 20µg i.n. days 14, 16, 18, 20)
Group 4: EtxB + OVA (i.p. OVA; i.n. EtxB 20µg + OVA 20µg i.n. days 14, 16, 18, 20)

Endpoints

Animals were sacrificed 24 hours after the last airway allergen challenge and sampling was a follows.

1. BAL: The trachea was exposed and cannulated. BAL was performed by two lavages with 0.8ml ice cold PBS, which were pooled and the volume and total cell number were determined. Cytospins were prepared for each sample by centrifugation of 50µl BAL fluid. These were fixed in acetone and stained with Diff Quick. Differential counts of 2 x 100 cells were performed classifying the cells as either neutrophils, eosinophils, lymphocytes or macrophages. Cell free lavage fluids were stored for later analysis. Thawed sterile BAL fluid was tested for the presence of cytokines (IL-4, IL-10, γIFN) by ELISA. Quantities of the cytokines were calculated using linear regression analysis.
2. Nasal wash: Reversal of the tracheal canula allowed 0.5ml PBS to be drawn through the nose to wash the nasal mucosa for antibodies. The volume was noted and the samples frozen for later antibody analysis.
3. Blood: Animals were bled by cardiac puncture under terminal anaesthesia. The blood was allowed to form a clot and the serum was removed following centrifugation. Serum samples were frozen for analysis later.
4. Antibodies: Nasal washes were analysed for the presence of anti-OVA IgA levels. Serum samples were analysed for the presence of anti-OVA total Ig, IgE, IgG1 and IgG2a levels. Antibody analysis was carried out by standard ELISA. Data was analysed by linear regression analysis.

Results

Figure 1. The effects of EtxB on total cell infiltration into the lung.
The data represent counts of total cells/BAL fluid recovered for each group. The data clearly show that injection of OVA in alum sensitizes Balb/c mice to lung cell infiltration following exposure to aerosolized OVA. Treatment with either EtxB alone or EtxB + OVA dramatically suppresses the allergic response in the BAL.

Figure 2. The effects of EtxB on total cell infiltration into the lung II.
The data show the results of analysing the numbers of individual leukocyte populations infiltrating the lungs after exposure to aerosolized OVA. Differential counts of cytospins prepared from BAL samples were used for the analysis. The results clearly indicate that EtxB treatment suppresses infiltration of each cell type into the lung. Importantly, the most dramatic difference is in the levels of eosinophils present in the BAL fluid. Eosinophils are the major cell population which respond to the presence of IgE in the lung and release inflammatory mediators.

Figure 3. Effects of EtxB treatment on the levels of OVA-specific IgE in BAL fluids.
Samples of BAL fluid from individual mice were analysed using doubling dilutions for the presence of anti-OVA IgE by specific ELISA. Linear regression analysis was used to determine endpoint titres, which are shown for each animal (squares) along with the median for the groups (bar). The data demonstrate that EtxB and EtxB + OVA can modulate local IgE levels in the lung.

Figure 4. Effects of EtxB treatment on the levels of cytokines in BAL fluids
The presence of IL-4, IL-10 and γIFN in individual BAL fluids from mice was determined by ELISA. The quantity of each cytokine present was calculated by linear regression analysis as compared to a standard curve obtained with recombinant cytokine. The data indicate that sensitzation with OVA in alum leads to an elevation in the quantities of IL-4 (the major cytokine associated with the generation of IgE). IL-10 and γIFN was detected in the negative controls as well as in the positive controls. Treatment with EtxB or EtxB + OVA dramtically suppressed the levels of IL-4 in BAL. EtxB may have raised the levels of IL-10 (a key regulatory cytokine) however the wide variation in the levels detected in individual mice precludes a strong conclusion being drawn on this point. Squares represent levels of cytokine in individual mice and the bar is the median for each group.

Figure 5. Effects of EtxB treatment on the levels of OVA-specific IgA in nasal washes
It is conceivable that EtxB may have prevented clinical asthma by switching the nature of the anti-OVA immune response away from a pro-IgE Th2 response and toward a mucosal IgA response. If this were the case, then it would be rpedicted that anti-OVA IgA levels would be raised in the nasal secretions. Analsysis of nasal anti-OVA IgA by specific ELISA revealed that EtxB treatment did not alter the levels of mucosal antibodies. In contrast, treatment with EtxB + OVA produced a substantial rise in anti-OVA IgA levels. This indicates that while both EtxB alone and EtxB + OVA were effective at reducing cell infiltration into the lung as well as altering local IgE and cytokine production, the mechanisms of immune modulation in the presence of the priming antigen are markedly different. Squares represent levels of cytokine in individual mice and the bar is the median for each group.

Figure 6. Effects of EtxB treatment on the levels of OVA-specific IgE in serum
Levels of anti-OVA IgE were measured in samples of serum by specific ELISA. A series of doubling dilutions of each serum sample was used in order to calculate endpoint titres using linear regression analysis. Squares represent levels of cytokine in individual mice and the bar is the median for each group. The data show that sensitization with OVA/alum leads to increased levels of anti-OVA IgE in the serum and that treatment with EtxB or with EtxB + OVA inhibits this.

Figure 7. Effects of EtxB treatment on the levels of OVA-specific IgG1 in serum
Serum levels of anti-OVA IgG1 were monitored since this IgG subclass is classically associated with a Th2 response of the type that also triggers allergy. Although IgG1 is not itself likely involved in the pathological processes underlying asthma, it is more abundant in serum and is therefore a good marker for the nature of the immune response. The data from analysing individual serum samples shows that OVA/alum sensitization triggers high levels of anti-OVA IgG1, treatment did not alter this. Squares represent levels of cytokine in individual mice and the bar is the mean for each group

Figure 8. Effects of EtxB treatment on the levels of OVA-specific IgG2a in serum.
Serum levels of anti-OVA IgG2a were monitored as this subclass is classically counter-regulated with IgG1. IgG1 is promoted by IL-4 and hence Th2, while IgG2a is promoted by γIFN and hence Th1. The data indicate that OVA/alum does give rise the production of IgG2a antibodies to OVA. However, it is noteworthy that the endpoints are approximately 2 logs lower than for IgG1. This is expected since alum and the model are associated with a Th2 dominant reaction. EtxB treatment appears to reduce levels of IgG2a to a certain extent. EtxB + OVA does not affect the levels of IgG2a.

Discussion

Taken together, the data clearly indicate that EtxB and EtxB + OVA are effective treatments in the OVA asthma model. All of the indicators in the lungs were favourably affected by EtxB treatment. EtxB reduced cell infiltration, particularly eosinophil infiltration. Further, EtxB reduced local IgE and associated IL-4. Analysis of other tissues revealed that the decrease in local IgE was associated with a more mild reduction in serum IgE, but not with changes to mucosal IgA and serum IgG1 levels. A slight reduction in serum IgG2a was noted. The findings also suggest that nasal EtxB triggers the activation of regulatory T cells which then enter sites of inflammation and suppress ongoing responses there.

Fig 1: The effects of EtxB on total cell infiltration into the lung

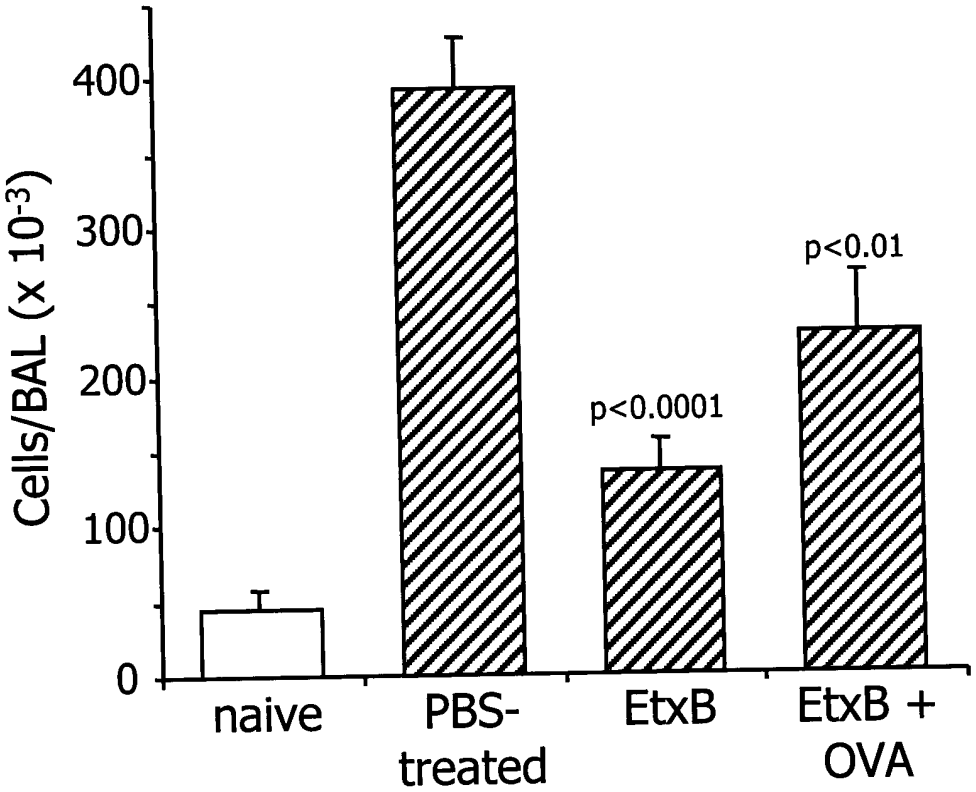

Fig 2: The effects of EtxB on cell infiltration into the lung

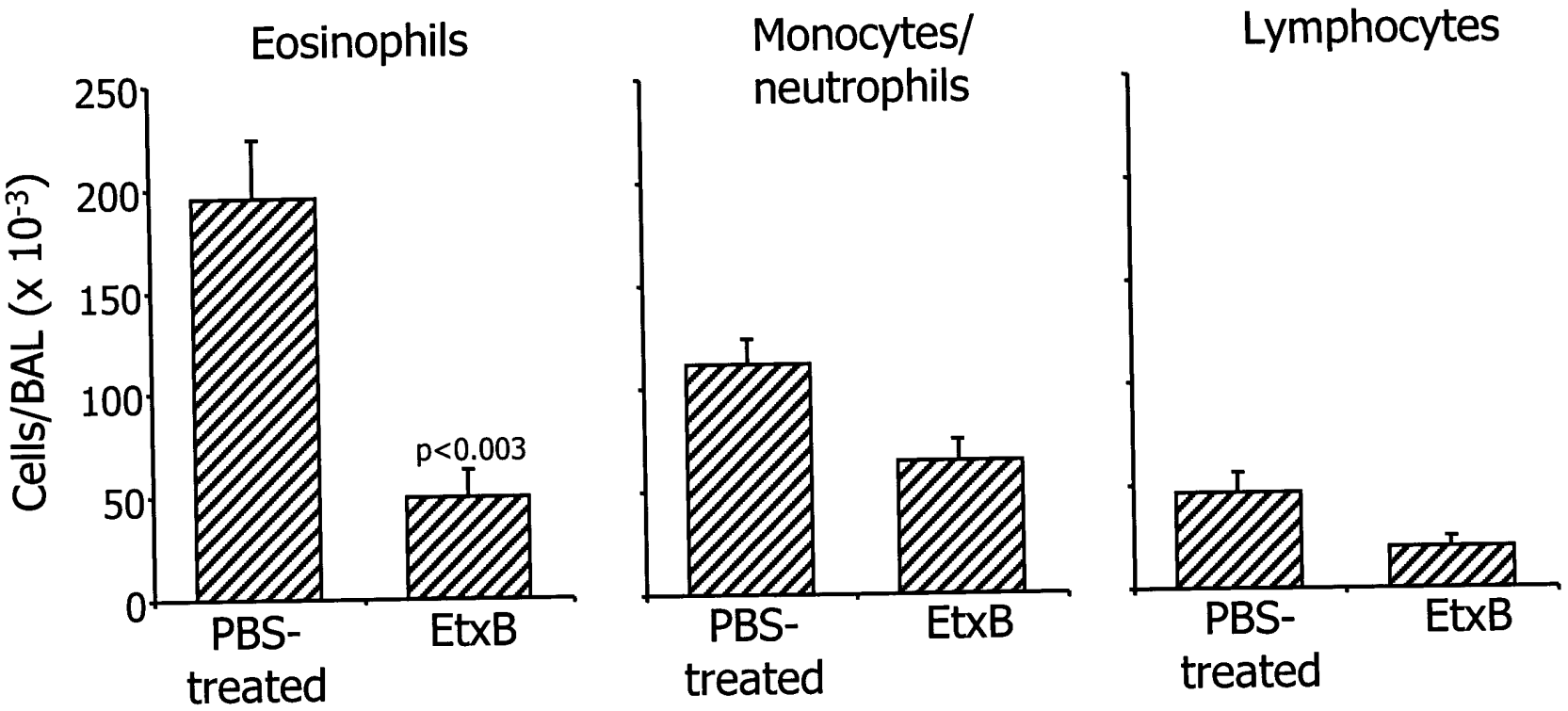

Fig 3: Effects of EtxB treatment on the levels of OVA-specific IgE in bronchoalveolar lavage fluids

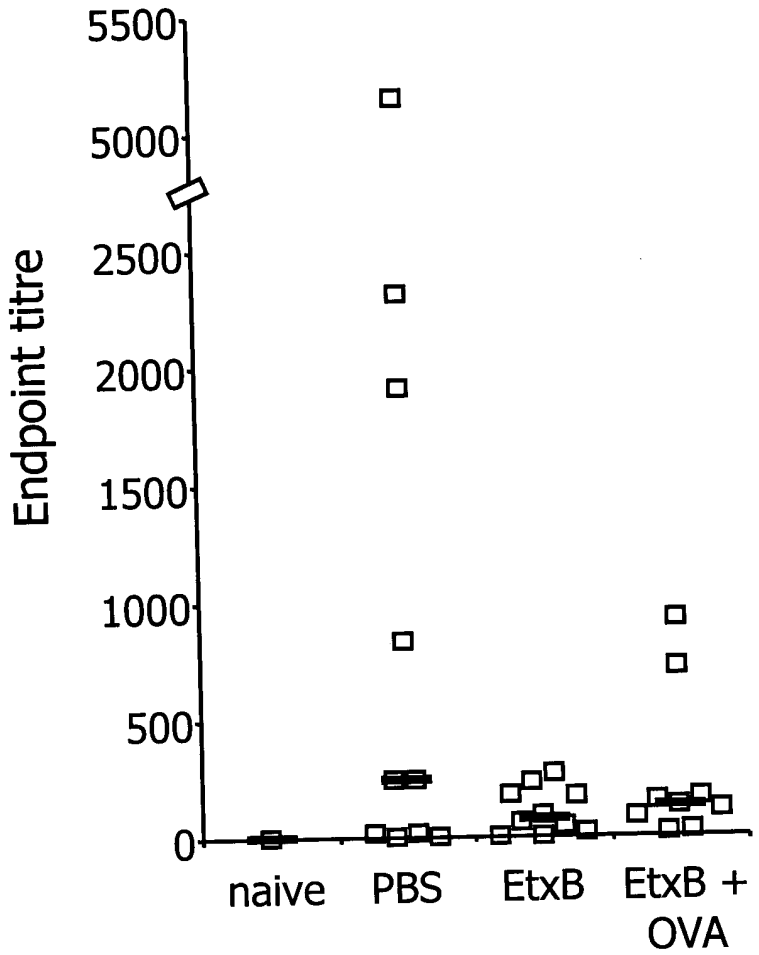

Fig 4: Effects of EtxB treatment on the levels of cytokines in bronchoalveolar lavage fluids

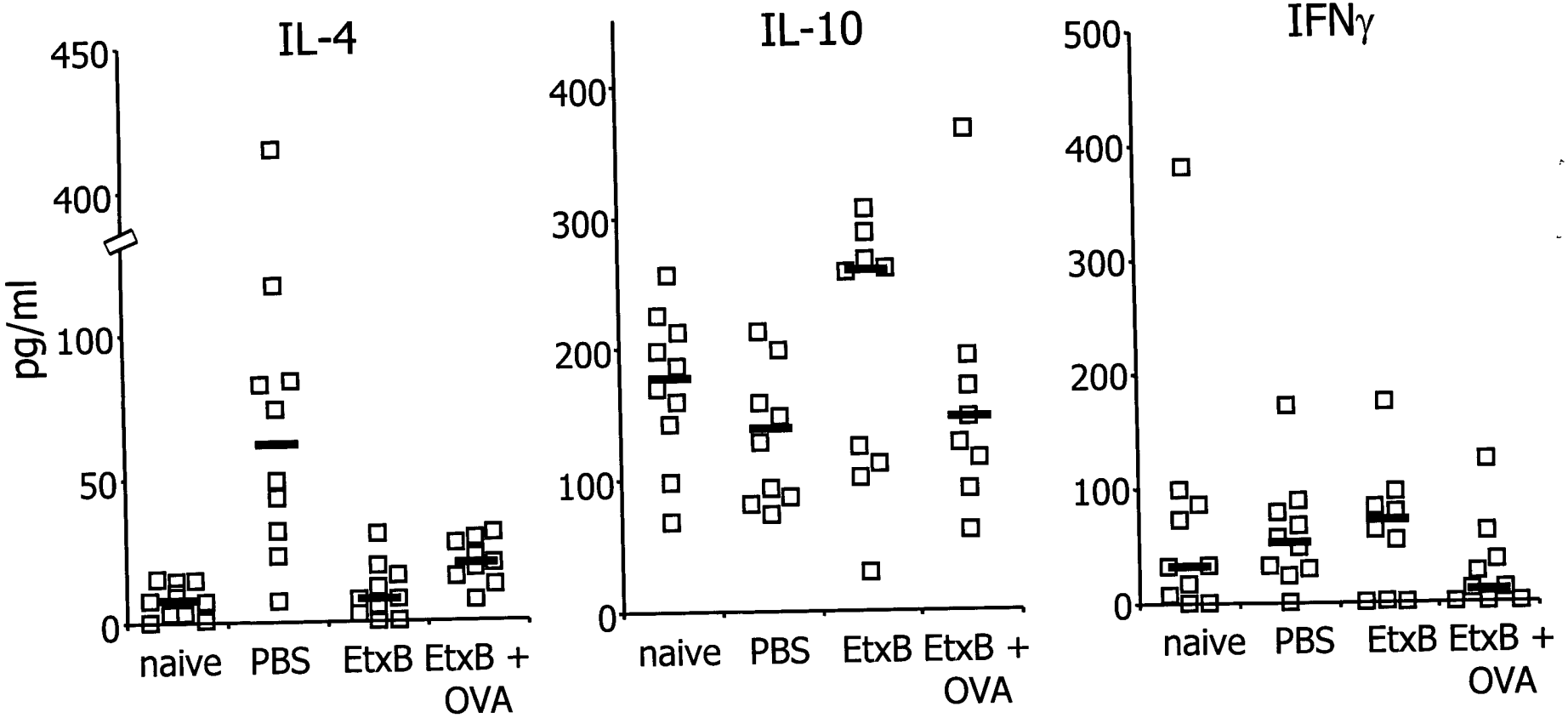

Fig 5: Effects of EtxB treatment on the levels of OVA-specific IgA in nasal washes

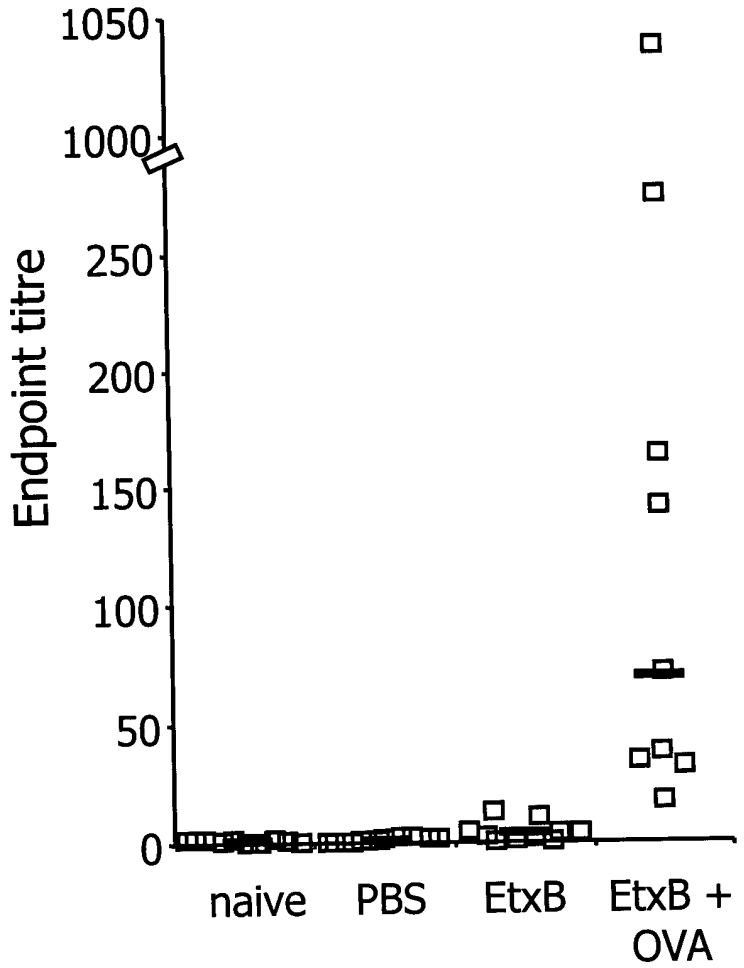

Fig 6: Effects of EtxB treatment on the levels of OVA-specific IgE in serum

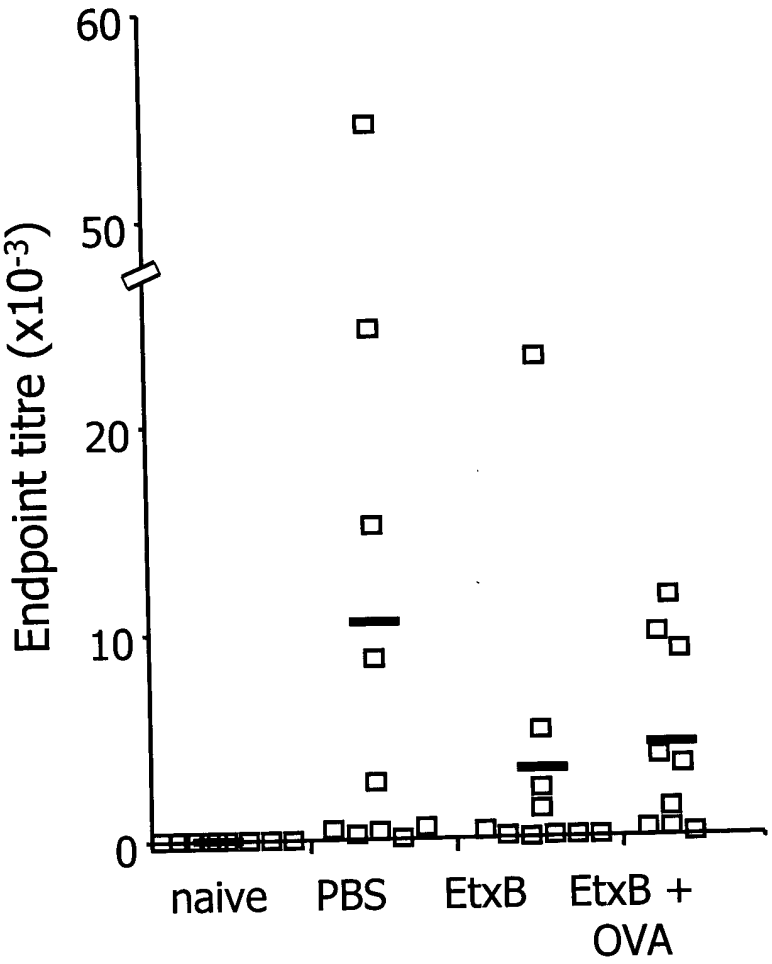

Fig 7: Effects of EtxB treatment on the levels of OVA-specific IgG1 in serum

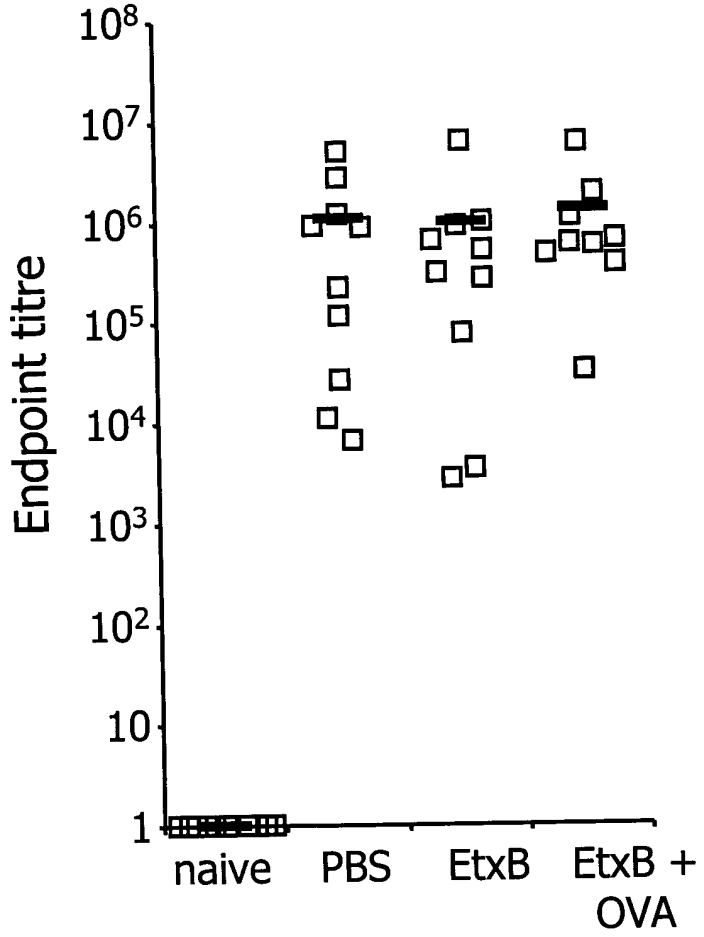

Fig 8: Effects of EtxB treatment on the levels of OVA-specific IgG2a in serum